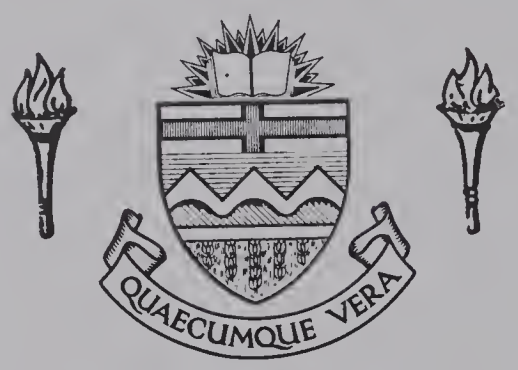

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THE UNIVERSITY OF ALBERTA

AN EVALUATION OF *CAREX* AND KENTUCKY BLUEGRASS HAYS GROWN ON A PARTIALLY DRAINED LAKE BOTTOM

by

© GLYN MICHAEL JOHN HORTON

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "An Evaluation of Carex and Kentucky Bluegrass Hays Grown on a Partially Drained Lake Bottom" submitted by Glyn Michael John Horton, B.Sc. (Agr.), in partial fulfilment of the requirements for the degree of Master of Science.

Abstract

The nutritive values of samples of *Carex* and Kentucky bluegrass grown on a partially drained lake bottom 50 miles west of Edmonton were assessed employing *in vivo* and *in vitro* techniques. Samples of oat straw and brome-fescue, grown in other locations, were employed as low and high quality control forages.

The phosphorus contents of the test forages were not adequate to meet the National Research Council (1970) nutrient requirements of beef cattle.

The *in vivo* digestibilities of the test forages were similar but the voluntary consumption of Kentucky bluegrass was 1.6 times that of *Carex*. There was a consistent trend for the *in vitro* dry matter and cellulose digestibilities of Kentucky bluegrass to be slightly higher than those of *Carex*.

Aqueous extracts of Kentucky bluegrass were equal or superior to those of brome-fescue hay in their ability to support the digestion of purified cellulose by rumen microorganisms *in vitro*. Sodium phosphate enhanced cellulose digestion in the presence of the aqueous extracts of the test forages but there was no response to phosphate when extracts of brome-fescue hay were employed. The digestion of cellulose in the presence of aqueous extracts of forages did not respond to the addition of sodium sulphate.

When the test forages were fed to cows, the level of trichloro-acetic acid-insoluble nitrogen and of total volatile fatty acids in the rumen fluid were 1.57 mg per ml and 8.65 m-equiv per 100 ml for *Carex* and 1.73 mg per ml and 8.76 m-equiv per 100 ml for Kentucky bluegrass. However, these values were 5.18 mg per ml and 11.17 m-equiv per 100 ml when

brome-fescue hay was fed. There were greater concentrations of n-butyric, iso-butyric, n-valeric and iso-valeric acids, which are required for cellulolytic rumen microorganisms, in the rumen fluid of cows fed Kentucky bluegrass than in the rumen fluid of the cows fed Carex. The concentrations of n-butyric, iso-butyric and iso-valeric acids were highest in the rumen fluid of cows fed brome-fescue hay.

It is suggested on the basis of results obtained in the present study, that the nutritive value of the Kentucky bluegrass was higher than that of the Carex. This greater value may have been related to its being a more favourable substrate for rumen fermentation than was Carex as well to as its greater acceptability.

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Introduction

As measured by animal performance, forages grown in the central and northern foothill regions of Alberta appear less productive than those grown in the recognized rangeland of the province. The purpose of this study was to study the quality of forages from a partially drained lake bottom 60 miles west of Edmonton. The main approach was to attempt to determine by laboratory procedures what factors that contribute to forage quality might be limiting in the samples selected.

The feeding value of herbage is dependent on the extent to which it is digested by the ruminant animal, the nature of products formed within the digestive tract and the level of voluntary intake. A considerable portion of the biological energy in forages is contained in the cell wall fraction. Within limits determined mainly by the degree and extent of lignification, ruminants are able to utilize this fraction with the aid of symbiotic microorganisms in the fore-stomach. The ability of the rumen microbes to digest the cell wall fraction has a large influence on the nutritive value of the forage.

Conventional digestion trials for forage evaluation are based upon animal performance. Laboratory evaluation techniques are essentially aimed at obtaining analytical data that predict the extent of biological degradation in the ruminant.

Review of Literature

The terms nutritive value and quality will be used synonymously throughout the text with respect to forages. For many years the *in vivo* digestibility trial has been the standard method for estimating forage quality. However, such trials are expensive and time consuming and they require large amounts of forage. Many laboratory methods have been suggested with which to estimate forage quality; three main approaches have emerged, 1) chemical analysis, 2) *in vitro* dry matter or cellulose digestibility techniques and, 3) solubility tests. Considerable interest has developed recently in the use of *in vitro* fermentation techniques for the evaluation of forage nutritive value.

Forage quality is a function of chemical composition, digestibility, the nature of products formed during digestion and voluntary intake (Barnes, 1965). The rate of voluntary intake is governed by acceptability, rate of digestion and rate of passage through the digestive tract (Crampton, 1957; Blaxter and Wilson, 1962). An aspect not generally accounted for by *in vitro* methods of forage evaluation is that the forage ingested by grazing animals may, due to selective grazing, differ markedly in botanical and chemical composition from herbage available (Galt *et al.*, 1969).

Analysis of Forages

The Weende system of proximate analysis has been used for the nutritive evaluation of feeds for many years. Attempts to correlate chemically determined nutrients with forage quality have not been very successful. Some reasons for this are that both the amounts and balance or imbalance of many nutrients affect the nutritive value of feeds. The failure of classical fractionation schemes to measure nutritive entities

has also been responsible for the limited success (Chalupa and McCullough, 1967).

Stallcup and Davis (1965) reported a highly significant correlation between crude protein and digestible protein (r = +0.95). Significant correlations were reported between crude protein and forage digestibility by Bowden and Church (1962) and between crude protein and digestible protein by Sullivan (1964). In 1958, Asplund et al. observed that the crude protein content of 11 hays, with a wide range in percent crude protein, was closely correlated with VFA yields and with dry matter digestion in vitro and in vivo.

As a group, the cell wall substances (cellulose, hemicellulose and lignin) compose 40 to 80 percent of the dry matter of forages, and have more significance nutritionally than do the more digestible cell contents in ruminant rations (Sullivan, 1969). The major portion of the cell wall complex consists of cellulose (Hansen, 1958, cited by Sullivan, 1969). Sullivan (1969) reported that the digestion coefficients of cellulose in 46 grasses ranged from 56.1 to 89.7 percent. Lignin, besides being highly indigestible itself, has an inhibitory influence on the digestion of other cell wall substances (Allinson, 1969). Van Soest (1965, 1969) has shown considerable variation to exist between species with regard to the relationships between lignin and in vivo parameters. The use of quantitative estimates of lignin as predictors of forage quality must consequently be limited. Sullivan (1969) reported that the cellulose content in grasses was highly correlated with both the digestibility of dry matter and its own digestibility.

Low concentrations of ether extract are found in roughages and it is therefore relatively unimportant as a source of energy. The three

vitamins most necessary to ruminants (Vitamins A, D and E) are lipid-soluble and are found in the ether extract fraction.

All the minerals except a small proportion of volatile materials are present in the ash of forages. The most important factors influencing the composition of the ash are the kind of forage and soil fertility (Beeson, 1941). The concentrations of the individual elements tend to diminish as the plants approach maturity (Pritchard et al., 1964). The interrelationships among certain minerals in the diet, as well as the actual amounts, govern the usefulness and also the harmful effects. The minerals in the diet must also meet the requirements of the microbial population in the rumen.

The following minerals have been found to perform essential functions in the body: phosphorus, calcium, magnesium, potassium, copper, manganese, zinc, selenium, iron, molybdenum, iodine, chlorine, sulphur, cobalt and sodium (Maynard and Loosli, 1969, p. 193). Hubbert et al. (1958 a) demonstrated the requirements and toxic levels of the following minerals for cellulolytic rumen bacteria: calcium, magnesium, copper, manganese, zinc, iron, sulphur and cobalt. In a later experiment, Hubbert et al. (1958 b) reported that potassium was also essential for in vitro cellulose digestion by rumen bacteria. Burroughs et al. (1951) demonstrated that phosphorus is required by rumen microbes for efficient cellulose digestion. In vitro cellulose digestion was increased from 34 to 80 percent when 80 mcg per ml of phosphorus were added to the fermentation medium (Anderson et al., 1956).

Hubbert et al. (1958 a) reported that sulphur, magnesium and calcium were the inorganic nutrients most frequently deficient in prepared fermentation media and extremely low levels of copper, zinc, cobalt and

boron depressed cellulose digestion *in vitro*.

In Vitro Digestibility Studies

(a) Dry Matter

The first reported use of an *in vitro* technique specifically for the quantitative estimation of forage digestibility was by Pigden and Bell in 1955. They observed a significant correlation between anthrone carbohydrate digestion *in vitro* and the digestion of organic matter of forages *in vivo*. In a study of hays of separate origin and different quality, Asplund *et al*. (1958) reported a pooled correlation coefficient of 0.71 between *in vivo* and *in vitro* digestible dry matter values.

Tilley *et al*. (1960) found the closest agreement between *in vivo* and *in vitro* dry matter digestibility with feeds of low digestibility that also had low protein contents. Further work revealed that a secondary digestion with the proteolytic enzyme pepsin resulted in higher correlations than incubations with rumen fluid alone. Twenty orchardgrass samples were studied using 'rumen fluid' and 'rumen fluid plus pepsin.' The correlation coefficient between *in vivo* and *in vitro* results for 'rumen fluid' was approximately 0.90 and for 'rumen fluid plus pepsin' 0.98. The pepsin stage reduced the standard error of the estimate of digestibility from approximately 4.0 to 2.0.

In later research using 130 samples of grass and 18 samples of clover and alfalfa of known *in vivo* digestibility (Y), Tilley and Terry (1963) established the regression equation, $Y = 0.99X - 1.01$ with a standard error of estimate of 2.31. The *in vitro* dry matter digestibility (X) was obtained from a 48 hr incubation with rumen fluid and an additional 48 hr incubation with pepsin.

Barnes (1966) reported that dry matter digestibility values obtained with an *in vitro* fermentation technique utilizing a 48 hr incubation period with rumen fluid plus a 24 hr incubation with pepsin, were more highly correlated (0.97) with *in vivo* digestible dry matter than were results obtained with various solubility methods and other *in vitro* fermentation techniques. In a study of 56 hays of known *in vivo* digestibility, Oh *et al.* (1966) reported that the dry matter digestibility values obtained by the two-stage Tilley and Terry method (1963), gave more reliable estimates of forage digestibility than did crude protein, acid-detergent fibre, acid-detergent lignin, cell-wall content, cellulose solubility in cupriethylene diamine, dry matter solubility in 1 N H_2SO_4 or *in vitro* cellulose digestion.

(b) Cellulose

Cellulose is a major constituent of forages which ruminants have the capacity to utilize through the action of microorganisms in the rumen. As a result, *in vitro* cellulose digestion has been used by several workers as a criterion for forage quality evaluation.

Barnett (1957) incubated 27 silage samples with whole rumen fluid, pooled from a number of sheep, for 70 hr and reported a reasonable degree of correlation between the *in vitro* cellulose digestion and the *in vivo* digestion of crude fibre. Reid *et al.* (1960) observed highly significant correlations between *in vitro* cellulose digestion and both *in vivo* digestible energy and the digestibility of dry matter *in vivo* with 124 forages from seven research stations. Hershberger *et al.* (1959) reported that *in vitro* cellulose digestibility values for 35 forages were more closely related to those for *in vivo* digestibility than were either crude protein or crude fibre contents. Correlation coefficients of 0.97

and 0.92 were obtained between *in vitro* cellulose digestibility and *in vivo* digestible cellulose and digestible energy, respectively.

Baumgardt *et al.* (1962) compared a number of laboratory methods as predictors of *in vivo* values. Eleven forages with a range in crude protein content from 7.12 to 22.90 percent were tested. *In vitro* cellulose digestion data had the highest correlations with *in vivo* dry matter, organic matter and energy digestibility values. The solubilities of forage cellulose in cupriethylene diamine and dry matter in 1 N H_2SO_4 were poorly related to *in vivo* measurements.

In Vitro Digestion Rate

Reid *et al.* (1959) stated that the main purpose of forages in ruminant diets is to provide energy. The nutritive value index, proposed by Crampton *et al.* in 1960, equated the quality of forage to the product of percent digestible energy times the relative intake. The relative intake was derived from the observed intake of a test forage, expressed relative to that of a standard forage and in relation to the metabolic weight of the animal. Crampton (1960) suggested that the significance of the relative intake of a forage as compared to its digestibility in determining the nutritive value index was approximately 70 percent vs. 30 percent, respectively.

Several workers have reported unequal lag periods during the *in vitro* digestion of cellulose in different forages with rumen microorganisms (Karn *et al.*, 1967; Warner, 1956; Hershberger *et al.*, 1959). Donefer *et al.* (1960) suggested that the prolonged lag periods associated with cellulose digestion could explain some of the factors affecting the level of intake. They proposed that *in vitro* cellulose digestibility measurements could be used in the prediction of forage intake and

digestible energy potential and examined the relationships between the *in vitro* cellulose digestibility of nine forages and relative intake, energy digestibility and nutritive value index. A highly significant correlation (0.91) was obtained between 12 hr *in vitro* cellulose digestibility values and those for nutritive value index. The regression equation obtained was Y = 1.31X - 7.8, where Y = nutritive value index and X = 12 hr *in vitro* cellulose digestibility. They proposed that the nutritive value index of forages could be predicted from values for 12 hr *in vitro* cellulose digestibility.

Water Extracts of Forages

In 1946, Elsden incubated a 10 percent (W/W) suspension of forage in water with cellulose and rumen liquor and reported that the fermentation of cellulose was accompanied by the production of acetic, propionic and butyric acids. Burroughs *et al.* (1950 a) prepared water extracts of alfalfa meal (17 percent protein) by adding enough water to make a thin gruel and pressing out the extract using a large hand-press. The digestion of ground corncobs *in vivo* was improved by the addition of the water extract of alfalfa and also by the addition of alfalfa ash to the diet. They concluded that alfalfa contained nutrients beneficial to the digestion of corncobs and that these nutrients were partially or totally soluble in water and in part or totally associated with the inorganic constituents contained in alfalfa hay.

In another digestion trial with cattle, Burroughs *et al.* (1950 b) added available nitrogen [$(NH_4)_2SO_4$], a complex mineral mix and an autoclaved water extract of cow manure, singly and in combination, to poor and high quality roughages. The cellulose in the good quality hay was digested efficiently without supplementation. However, the additions

of complex minerals and manure extract along with available nitrogen greatly increased cellulose digestion in poor quality roughages. They concluded that the efficiency with which a roughage is digested is dependent upon the presence or absence in the forage of essential nutrients which are required for maximum functioning of cellulolytic bacteria.

Marquardt and Asplund (1964) compared 14 forages on the assumption that the water-soluble fraction represented that portion of the forage most readily available to cellulolytic rumen microorganisms. Purified cellulose plus aqueous extracts of forages was digested by rumen microorganisms in all-glass fermentation vessels. This method was not considered sensitive enough for routine forage evaluation. They then added urea, phosphate and sulphate singly and in combination to the purified cellulose, forage extracts and rumen microorganisms. The highest rates of cellulose digestion were observed when extracts were supplemented with urea, phosphate and sulphate. They concluded that, with the exception of phosphate, sulphate, and, in some cases, nitrogen, an adequate supply of nutrients required for cellulose digestion by cellulolytic microbes was usually present in the aqueous extracts of forages.

Volatile Fatty Acid Production

In 1884 von Tappeiner (cited by Marston, 1948) reported that gas (methane or hydrogen and carbon dioxide) and acids (assumed to be acetic and butyric) were found when cellulose was incubated with rumen fluid. He concluded that rumen bacteria were responsible for cellulose fermentation and hypothesized that volatile acids were absorbed and oxidized by the host to supply its energy needs. This theory was disputed

for many years, but was later supported by workers such as Phillipson (1942), who stressed the nutritional importance of the VFA present in the rumen. The main body of VFA production in the rumen comes from the carbohydrates present in the feed and plays an important role in the energy metabolism of ruminants (Hungate, 1966).

Phillipson (1942) observed that mangolds and cabbages supported a more rapid fermentation rate than did an all-hay diet. He suggested that the composition of the diet could influence the rumen population and pattern of VFA produced in the rumen. The relationship between the composition of the diet and the individual fatty acids in the rumen liquor was examined by Bath and Rook (1965). Those diets that showed the highest proportions of acetic acid, about 70 percent, were the all-roughage diets of hay or silage. Those that showed the lowest proportions of acetic acid, 55-60 percent, were the succulents and other bulky foods lacking in the physical quality of fibrousness.

Chalupa and McCullough (1967) investigated the relationships between certain constituents of Gulf Ryegrass and the molar percents of fatty acids produced intraruminally. Data suggested that factors in plants which caused lowered digestibility also produced acid ratios high in acetate. Production of butyrate and propionate was enhanced by plant factors favouring high digestibility.

Asplund et al. (1958) compared total VFA production from 11 hays in all-glass in vitro fermentation vessels. Rumen fluid was collected from sheep fed hay and from sheep fed straw. Coefficients of correlation between both VFA production and dry matter loss in vitro and dry matter digestibility in vivo were either significant or highly significant. The nature of the diet influenced total VFA production in that significantly

higher fatty acid levels were found in the rumen fluid from sheep fed hay than in that from sheep fed straw.

Bath and Rook (1963) observed higher total VFA concentrations in the rumen with increased hay intakes. The amounts and proportions of the individual acids absorbed have been demonstrated to influence the value of the diet for fattening (Armstrong and Blaxter, 1957).

Experiments at The University of Alberta

Analysis of Forages

Introduction

Several attempts have been made to correlate chemically determined nutrients with the overall quality of forages. In many cases these correlations have not been satisfactory. This is mostly because both the amounts and balance or imbalance of several nutrients affect the digestion and nutritive value of feeds. The inadequacy of classical chemical fractionation schemes to measure nutritive entities has contributed to the limited success (Van Soest, 1967). The evaluation of forages for their nutritive value cannot be accomplished with certainty by chemical analysis but some approximations to nutritive value are possible by this means (Sullivan, 1964).

Experimental

Test Forages

Forage samples from the Cormie Ranch Ltd. were investigated. The ranch is situated near the town of Tomahawk, about 60 miles west of Edmonton. Dr. A.W. Bailey from the Department of Plant Science assisted in the selection of two sampling areas and determined the botanical composition of the swards on these sites. The legal description of the sampling areas and botanical composition of the swards on each are presented in Table 1.

Both sampling areas were located in a large, partially drained lake bottom, approximately 8,100 acres in extent. The location from which *Carex* samples were taken was poorly drained, with surface water visible in some parts. Kentucky bluegrass was harvested from a site in

Table 1

Legal description of sample areas and botanical composition of swards in these locations.

Sward	Location	Percent botanical composition		
		Carex vesicaria	Poa pratensis	Beckmannia syzigachne
Carex	NE 12-51-5-W5	100	0	0
Kentucky bluegrass	SW 11-51-5-W5	3	95	2

the lake bottom that had been well drained for a number of years. One ton of each hay was cut and baled in the early bloom stage under good harvesting conditions, and transported to The University of Alberta, Edmonton Research Station.

Soil Type

Soil test information for several parts of the lake bottom area was obtained from Mr. D. Laverty, Director of the Agricultural Soil and Feed Testing Laboratory, Edmonton. Organic soils are present in the lake bottom area and are generally low in nitrogen, phosphorus and potassium. The soils are strongly acid in reaction and show salt accumulation in most areas.

Control Forages

Oat straw from the Edmonton area was used as the lower standard in in vitro fermentation studies. Samples were taken from a single bale.

Excellent quality brome-fescue hay from The University of Alberta, Edmonton Research Station, harvested in the early bloom stage, was selected as the upper standard.

Analysis of Forages

Moisture, protein, ether extract, gross energy and ash analyses were performed using the methods recommended by the Association of Official Agricultural Chemists (1965). Cellulose was determined using the Crampton and Maynard (1938) method, slightly modified as follows. Acid digestion was conducted in 90 ml pyrex glass centrifuge tubes which were immersed in boiling water for a period of 30 min (replacing the original 20 min refluxing period). Phosphorus was determined by the ammonia-metavanadate method outlined by Jackson (1958). Calcium, magnesium potassium, copper, manganese, zinc, iron and molybdenum in the forages were determined by the procedure described by David (1962) using a Techtron Atomic Absorption Spectrophotometer, Model AA-4. The analyses were carried out in quadruplicate.

The selenium analysis was carried out by the Soils Section of the Canada Department of Agriculture, Research Branch in Lacombe, Alberta.

Results

Results of the analyses of forages harvested in 1969 and investigated in the present study are presented in Table 2.

The protein contents of oat straw, Carex, Kentucky bluegrass and brome-fescue were 2.8, 11.2, 11.5 and 21.5 percent, respectively.

The highest proportions of cellulose were found in oat straw, 39.5 percent, the lowest in brome-fescue, 23.5 percent. Interjacent amounts of cellulose were present in Carex, 31.5 percent, and Kentucky bluegrass, 29.4 percent.

The concentrations of ether extract in oat straw, Carex, Kentucky bluegrass and brome-fescue were 1.0, 0.8, 1.1 and 2.4 percent, respectively.

Table 2

Composition of forages evaluated*.

	Oat straw	Carex	Kentucky bluegrass	Brome-fescue
Moisture, %	9.4	13.4	11.6	10.9
Protein, %	2.8	11.2	11.5	21.5
Cellulose, %	39.6	31.5	29.4	23.5
Ether extract, %	1.0	0.8	1.1	2.4
Gross energy, Kcal/gm	4.5	4.5	4.6	4.6
Ash, %	8.5	8.0	7.1	9.5
P, %	0.05	0.05	0.06	0.13
Ca, %	0.22	0.44	0.18	0.40
Mg, %	0.52	0.88	0.57	0.66
K, %	2.2	1.8	2.3	2.0
Cu, ppm	2.7	8.9	12.1	8.7
Mn, ppm	4.4	257	402	36
Zn, ppm	22.5	66.5	77.3	32.0
Se, ppb	58	36	36	97
Fe, ppm	275	496	431	337
Mo, ppm	<1.0	<1.0	<1.0	<1.0

* Harvested in 1969.

Similar gross energy values were found in oat straw, Carex, Kentucky bluegrass and brome-fescue - 4.5, 4.5, 4.6 and 4.6 Kcal/gm, respectively.

The ash contents of oat straw, Carex, Kentucky bluegrass and brome-fescue were 8.5, 8.0, 7.1 and 9.5 percent, respectively.

Lower levels of phosphorus were found in oat straw, 0.05 percent, Carex, 0.05 percent, and Kentucky bluegrass, 0.06 percent, than in brome-fescue, 0.13 percent. Lesser amounts of calcium were present in oat straw, 0.22 percent, and Kentucky bluegrass, 0.18 percent, than in Carex, 0.44 percent, or brome-fescue, 0.40 percent.

Similar amounts of magnesium - 0.52, 0.88, 0.57 and 0.66 percent, potassium - 2.2, 1.8, 2.3 and 2.0 percent and molybdenum - <1.0 ppm were found in oat straw, Carex, Kentucky bluegrass and brome-fescue, respectively.

Lower levels of copper were present in oat straw, 2.7 ppm, than in Carex, 8.9 ppm, Kentucky bluegrass, 12.1 ppm, or brome-fescue, 8.7 ppm.

Higher amounts of manganese were found in Carex and Kentucky bluegrass - 257 and 402 ppm, respectively, than in oat straw or brome-fescue - 4.4 and 36 ppm, respectively.

The concentrations of zinc and iron were higher in Carex - 66.5 and 496 ppm, respectively, and Kentucky bluegrass - 77.3 and 431 ppm, respectively, than in brome-fescue - 32.0 and 337 ppm, respectively, or oat straw - 22.5 and 275 ppm, respectively.

Lower concentrations of selenium were found in Carex and Kentucky bluegrass - 36 and 36 ppb, respectively, than in oat straw, 58 ppb, or brome-fescue, 97 ppb.

Discussion

Forages should contain at least 5.9 percent protein for dry pregnant mature cows and as much as 13.9 percent protein for 300 lb young bulls for growth and maintenance (NRC, 1970). Carex and Kentucky bluegrass were harvested in the early bloom stage when protein levels were relatively high.

The phosphorus requirements in beef rations range from 0.16 percent for mature cattle to 0.43 percent for young animals (NRC, 1970), thus the phosphorus in Carex and Kentucky bluegrass would only have met approximately one-third of the requirements of mature cattle.

The NRC (1970) requirements for calcium in beef rations range from 0.16 percent for mature cattle to 0.60 percent for young cattle fed a concentrate ration for rapid weight gains, thus the calcium in Carex would have met the requirements of most classes of beef cattle. However, the lower levels in Kentucky bluegrass were only adequate for mature cattle.

The magnesium requirement of farm animals for growth is in the order of 0.06 percent of the dry ration, assuming that the phosphorus and calcium intakes are adequate but not excessive (Maynard and Loosli, 1969, p. 179). The test and control forages contained approximately ten times the required level of magnesium.

With average rates of consumption, forages containing 0.39 percent of potassium met the requirements for grazing cattle (Sullivan, 1969). Adequate amounts of potassium were present in oat straw, Carex,

Kentucky bluegrass and brome-fescue - 2.2, 1.8, 2.3 and 2.0 percent, respectively.

The requirements and toxic levels of some trace minerals for cattle as listed by Maynard and Loosli (1969, p. 193) are presented in Table 3. In terms of the above reference, the copper levels in Carex, 8.9 ppm, Kentucky bluegrass, 12.1 ppm and brome-fescue, 8.7 ppm were adequate. The low concentration of copper in oat straw, 2.7 ppm, is typical of this forage.

The manganese levels, 16-25 ppm, were adequate in all roughages, 36-402 ppm, except oat straw, 4.4 ppm. Cunningham et al. (1966) reported that body weight gains and feed intakes in calves were not affected by manganese levels up to 2,400 ppm in the diet.

Adequate, non-toxic concentrations of zinc and selenium were present in all four roughages. Sullivan (1969) reported that molybdenum levels of 0.5 to 3 ppm in the feed were not toxic. The feeds in the present study contained less than 1 ppm of molybdenum.

Table 3

Requirements and toxic levels of some trace mineral elements in cattle rations.

Element	Requirements	Toxic levels
	ppm	ppm
Cu	5-7	115
Mn	16-25	2,000
Zn	9	900->1,200
Se	0.1	3-4

Experiment 1

In Vivo Digestibility Trials

Introduction

In the majority of literature published on the evaluation of feed quality, apparent digestibility has been used as the basis of comparison (Maynard and Loosli, 1969, p. 331). For a particular dietary component, the amount of that component in the feed minus the amount in the feces provides an estimate of the amount of the component apparently digested.

Experimental

Hatton and Owen (1969) investigated the accuracy of digestibility trials in the determination of forage digestibility using different periods of collection. They concluded that 3-day collection periods with 5 cows were as accurate as 7-day collection periods using 3 cows. In the present study, digestibilities of *Carex* and Kentucky bluegrass were determined while the *in vitro* digestion experiments were in progress, and as rumen fluid was collected once per week, collection periods of longer than 5 days were not possible.

The test animals were fed the test rations at least 4 weeks before the start of each trial. Feed was offered twice daily at 9 AM and 4 PM. Unconsumed hay was weighed prior to the morning feeding and 10 percent more hay per day was offered than was consumed the previous day. After the first trial, the diet of the heifer fed *Carex* was changed to Kentucky bluegrass, and that of the heifer fed Kentucky bluegrass was changed to *Carex*. The first trial was conducted over a 4 day period, the second over 5 days.

The feces were collected and weighed four times daily. Five percent by weight of each collection was retained and stored at -3°C. Samples were taken out of storage, thawed and mixed thoroughly prior to analysis. The feces were dried in a forced-draft oven for 48 hr at 70°C and ground in a laboratory mill through a 20 mesh screen.

Dry matter, nitrogen, and gross energy were determined on the fecal samples by AOAC (1965) methods. Cellulose was determined by the modified Crampton and Maynard (1938) method described under Analysis of Forages.

Results

The mean values for the two trials are presented in Table 4. Consumption of Kentucky bluegrass was 1.6 times that of *Carex*. Dry matter, cellulose, protein, and gross energy in Kentucky bluegrass were more digestible than those in *Carex*. With only one degree of freedom, none of the differences observed in Table 4 proved to be significant ($P < 0.05$).

Table 4

Voluntary consumption and *in vivo* digestibility of *Carex* and Kentucky bluegrass.

Roughage	Intake	Digestibility			
		Dry matter	Cellulose	Protein	Gross energy
	kg/day	%	%	%	%
Carex	5.6	55.0	58.3	44.0	54.3
Kentucky bluegrass	8.9	57.4	62.8	46.4	57.3

Discussion

The protein content of forages is positively correlated to dry matter digestibility (Asplund *et al.*, 1958) and the cellulose content is negatively correlated to dry matter digestibility (Sullivan, 1964). Further, Blaxter and Wilson (1962) observed that dry matter digestibility was related to voluntary consumption. The protein content of *Carex*, 11.2 percent, was similar to that of Kentucky bluegrass, 11.5 percent, and similar amounts of cellulose were found in both *Carex*, 31.5 percent, and in Kentucky bluegrass, 29.4 percent (Table 4, p. 20). It is unlikely that either the protein or the cellulose contents in the test forages were *per se* related to the differences in voluntary intake. These *in vivo* results will be discussed later in relation to *in vitro* digestion results, the amounts of trichloroacetic acid-insoluble nitrogen in rumen fluid, VFA production and in the General Discussion.

Experiment 2

In Vitro Digestion Studies

Introduction

The nutritive value of forages is determined by the chemical composition and digestibility (Barnes, 1965). Asplund *et al.* (1958) and Tilley and Terry (1963) reported that reliable estimates of forage nutritive value were obtained from *in vitro* dry matter digestibility values; Johnson *et al.* (1964) predicted forage quality from *in vitro* cellulose digestibility data. *In vitro* digestibility values for dry matter and cellulose were determined in the selected samples of oat straw, *Carex*, Kentucky bluegrass and brome-fescue as estimates of their nutritive value.

Experimental

Experimental animals

Two Hereford heifers and one mature Jersey cow with permanent rumen fistulae served as sources of rumen fluid (Appendix 1). The animals were maintained at The University of Alberta, Edmonton Research Station. The Jersey cow was fed brome-fescue hay, one Hereford *Carex* and the other Kentucky bluegrass. The experiment was repeated four times, and after the third trial the rations fed to the Herefords were switched. The animals were fed twice daily at 9 AM and 4 PM. Prior to each feeding, unconsumed hay was weighed and discarded. All experimental animals had free access to calcium phosphate (20.5 percent phosphorus, 18.5 percent calcium) and iodized salt in the ratio of 1:1. Water was available *ad libitum* from automatic water bowls.

Inoculum collection

Feed and water were removed at 5 PM on the day prior to the

collection of rumen fluid. The animals were offered their respective diets for 1 hr at 6 AM on the collection day. The hay was then removed and 3 litres of water offered. Rumen fluid was collected at 9 AM. Rumen contents were manually removed from different parts of the rumen and squeezed through four layers of cheese-cloth in a squeeze press. The rumen liquor from each animal was collected in a separate thermos flask, previously warmed to 40°C, and taken to the laboratory as quickly as possible.

In Vitro fermentation method

Several workers have reported that the two-stage in vitro technique of Tilley and Terry (1963) is one of the most reliable in vitro techniques for the estimation of in vivo digestibility (Oh et al., 1966; Barnes, 1966, 1969). The test hays, Carex and Kentucky bluegrass, and the control roughages, oat straw and brome-fescue, were evaluated by this method.

Forage samples were dried in a forced-draft oven for 24 hr at 100°C and ground in a Christy and Norris 8-in laboratory mill fitted with a 1 mm screen. The buffer solution was made up according to the formula of McDougall (1948) for 'synthetic-saliva.' The $CaCl_2$ was added last and the solution thoroughly saturated with CO_2 at 39°C until it became clear. The pepsin solution was prepared by dissolving 2.0 g of 1:10,000 pepsin (National Biochemicals Corp.) in 850 ml of demineralized water; 100 ml of a 1 N HCl solution was then added and the solution made up to a final volume of 1 liter. Ninety ml polypropylene centrifuge tubes served as fermentation vessels and were capped with rubber stoppers fitted with Bunsen gas release valves. A constant temperature of 39°C was maintained in a thermostatically controlled water bath. The

incubation period was divided into two 48 hr stages.

First stage, rumen liquor digestion

Quadruplicate samples of approximately 0.5 g of each forage were accurately (0.1 mg) weighed into the fermentation tubes. The substrates were then inoculated with 10 ml of strained, whole rumen fluid in 40 ml of the buffer solution. The tubes were thoroughly gassed with CO_2, capped with rubber stoppers fitted with Bunsen valves, and incubated for 48 hr in the dark at 39°C. To ensure a uniform inoculation throughout each trial, buffer solution and rumen fluid were mixed in a large glass bottle, gassed continually with a stream of CO_2 and agitated with a magnetic stirrer. Fifty ml samples were siphoned off and randomly distributed among the fermentation vessels. The pH was maintained at 6.7 to 6.9 by the addition of 1 N Na_2CO_3.

Second stage, pepsin digestion

At the end of the rumen liquor digestion stage, microbial activity was checked by the addition of 1 ml of 5 percent $HgCl_2$; 2 ml of 1 N Na_2CO_3 were added to improve sedimentation. The tubes were then centrifuged at 1,500 X g for 15 min and the supernatant fluid poured off. Fifty ml of freshly prepared pepsin solution were added to each tube, the contents mixed thoroughly and the fermentation tubes returned to the water bath at 39°C for a further 48 hr. At the end of this period, the tubes were centrifuged for 15 min at 1,500 X g and the supernatant fluid discarded.

Analysis

The residue in the fermentation tubes was quantitatively transferred to 90 ml pyrex glass centrifuge tubes, washed once with distilled water and re-centrifuged as above. The supernatant fluid was

discarded and the residue dried to a constant weight in a vacuum oven at 95°C.

Dry matter digestibility was calculated as the percent dry matter loss during the *in vitro* fermentation. Blanks were determined by digesting 50 ml of the rumen fluid - buffer mixture in fermentation tubes. After the digestion period, the blanks were centrifuged, washed and dried in the vacuum oven as described previously.

$$\text{DM dig., \%} = 100 - \frac{(\text{DM before dig.} + \text{DM blank} - \text{DM after dig.}) \times 100}{\text{DM before dig.}}$$

Cellulose digestibility was calculated as the net loss of cellulose during incubation. The forages, residual dry matter after digestion and blanks were analyzed for cellulose by a modification of the Crampton and Maynard (1938) technique as described under forage analysis.

$$\text{Cell. dig., \%} = 100 - \frac{(\text{cell. before dig.} + \text{cell. in blank} - \text{cell. after dig.}) \times 100}{\text{cell. before dig.}}$$

Values for dry matter and cellulose digestibility were expressed on a dry matter basis.

Separate samples of the test and control roughages were inoculated with whole rumen fluid from each of three animals fed *Carex*, Kentucky bluegrass or brome-fescue. Each fermentation was in quadruplicate and the trial repeated four times. Data in Tables 5, 6, 7 and 8 represent the means of 16 values obtained from four *in vitro* digestion trials.

Standard errors, analysis of variance and mean comparisons by Duncan's multiple range test were carried out according to the methods described by Steel and Torrie (1960). The computations were done on an IBM 360/67 computer.

In Vitro Dry Matter Digestion

Results

The data in Table 5 indicate the ability of each inoculum to digest the dry matter of each of the four substrates. Both Carex and Kentucky bluegrass inocula digested the dry matter in oat straw significantly less ($P < 0.05$) and that in brome-fescue significantly more than the dry matter in either Carex or Kentucky bluegrass. The digestibility values for dry matter in the test forages, Carex and Kentucky bluegrass, did not differ significantly from each other for either Carex or Kentucky bluegrass inocula. No significant differences were observed in the digestibility of dry matter in the oat straw, Carex or Kentucky bluegrass samples fermented with brome-fescue inoculum. However, brome-fescue inoculum digested the dry matter in brome-fescue significantly more than it did that in the other three substrates. On the basis of the pooled data in Table 5, the digestibilities of dry matter in oat straw, 42.7 percent, Carex, 49.4 percent, Kentucky bluegrass, 54.5 percent, and brome-fescue, 73.3 percent, differed significantly from each other.

Differences in the activities of the three inocula on a given substrate are summarized in Table 6. The dry matter in oat straw was significantly more digestible ($P < 0.05$) when fermented with brome-fescue inoculum than when incubated with either Carex or Kentucky bluegrass inoculum.

The only other significant result in the inocula-substrate comparisons in Table 6 is that which shows that, basis the pooled data, the rumen fluid from the cow fed brome-fescue gave the highest dry matter digestibility values. There is a consistent trend in Table 6 to suggest

Table 5

Within-inocula differences in *in vitro* dry matter digestibility of control and test forages.

Inoculum	Digestibility of dry matter, Substrate: Oat straw Mean %	SE	Carex Mean %	SE	Kentucky bluegrass Mean %	SE	Brome-fescue Mean %	SE
Carex	34.0[a]	±1.90	47.4[b]	±2.54	51.5[b]	±1.33	72.1[c]	±1.60
Kentucky bluegrass	41.3[a]	±1.85	49.8[b]	±1.25	54.6[b]	±0.81	73.3[c]	±0.57
Brome-fescue	52.8[a]	±2.96	51.3[a]	±1.92	57.6[a]	±2.56	74.3[b]	±2.11
Pooled data	42.7[a]	±1.73	49.4[b]	±1.14	54.5[c]	±1.04	73.3[d]	±0.89

[abcd]Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Table 6

Between-inocula differences in *in vitro* dry matter digestibility of control and test forages.

Substrate	Digestibility of dry matter, Inoculum: Carex Mean %	SE	Kentucky bluegrass Mean %	SE	Brome-fescue Mean %	SE
Oat straw	34.0[a]	±1.90	41.3[a]	±1.85	52.8[b]	±2.96
Carex	47.4[a]	±2.54	49.8[a]	±1.25	51.3[a]	±1.92
Kentucky bluegrass	51.5[a]	±1.33	54.6[a]	±0.81	57.6[a]	±2.56
Brome-fescue	72.1[a]	±1.60	73.3[a]	±0.57	74.3[a]	±2.11
Pooled data	51.2[a]	±1.96	54.7[a]	±1.54	59.0[b]	±1.65

[ab]Means within each row bearing different superscripts differ significantly ($P < 0.05$).

that the rumen fluid from the cow fed brome-fescue gave the highest dry matter digestibility values and that the rumen fluid from the heifer fed Carex gave the lowest dry matter digestibility values.

Discussion

There was a trend for the digestibility of the dry matter in Kentucky bluegrass to be higher than that for Carex over all inocula, but the differences were significant only in the case of the pooled data - 54.5 vs. 49.4 percent (Table 5). The digestibility of Kentucky bluegrass dry matter with Kentucky bluegrass inoculum, 54.6 percent, was significantly ($P < 0.05$) higher than the digestibility of dry matter in Carex with Carex inoculum, 47.4 percent. In both cases the rumen microbes were adapted to the feeds and therefore represented climax populations.

Tilley and Terry (1963) established the regression equation $Y = 0.99X - 1.01$, where Y = in vivo digestibility and X = in vitro dry matter digestibility. When the in vitro dry matter digestibility values of Carex with Carex inoculum, 47.4 percent, and Kentucky bluegrass with Kentucky bluegrass inoculum, 54.6 percent, are substituted in the above regression equation, the predicted in vivo dry matter digestibilities are 45.9 and 53.0 percent, respectively. The predicted dry matter digestibility of brome-fescue from in vitro dry matter digestibility data, 74.3 percent, is 72.6 percent.

Tilley and Terry (1963) reported that reliable estimates of forage quality could be obtained from in vitro dry matter digestibilities. On the basis of the pooled results for dry matter digestibility (Table 5) the nutritive value of oat straw would be estimated to be the lowest, Kentucky bluegrass

more nutritious than Carex, and the nutritive value of brome-fescue the highest.

In Vitro Cellulose Digestion

Results

The data in Table 7 indicate the ability of each inoculum to digest the cellulose in each of the four substrates. With both Carex and Kentucky bluegrass inocula, the cellulose in oat straw was digested significantly less and that in brome-fescue significantly more than the cellulose in either of the test forages. Brome-fescue inoculum supported significantly higher levels of digestion of cellulose in brome-fescue than in oat straw, Carex or Kentucky bluegrass. The pooled data confirm that for the three sources of inocula, the levels of digestibility of the cellulose in Carex and Kentucky bluegrass were the same; the cellulose in oat straw was significantly less and that in brome-fescue significantly more digestible than that in the test forages.

Differences in the cellulolytic activities of the three inocula on each substrate are summarized in Table 8. The digestibility values for oat straw cellulose obtained with each of the inocula were different from each other at the 5 percent level of significance. No significant differences attributable to inoculum source were observed in the digestibility values for Carex, Kentucky bluegrass or brome-fescue celluloses. When data from the four substrates were pooled for each inoculum the results showed that the cellulolytic acitivity of Carex inoculum was significantly less than that of either Kentucky bluegrass or brome-fescue inoculum.

Table 7

Within-inocula differences in in vitro cellulose digestibility of control and test forages.

Inoculum	Digestibility of cellulose: Substrate: Oat straw Mean %	SE	Carex Mean %	SE	Kentucky bluegrass Mean %	SE	Brome-fescue Mean %	SE
Carex	14.2[a]	±1.78	36.8[b]	±4.35	35.6[b]	±2.34	61.9[c]	±3.48
Kentucky bluegrass	25.6[a]	±3.24	43.5[b]	±2.66	43.0[b]	±1.84	64.5[c]	±1.73
Brome-fescue	39.2[a]	±3.48	43.2[a]	±2.73	44.7[a]	±1.80	61.0[b]	±0.93
Pooled data	26.3[a]	±2.23	41.1[b]	±1.94	41.1[b]	±1.28	62.5[c]	±1.58

[abc]Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Table 8

Between-inocula differences in in vitro cellulose digestibility of control and test forages.

Substrate	Digestibility of cellulose: Inoculum: Carex Mean %	SE	Kentucky bluegrass Mean %	SE	Brome-fescue Mean %	SE
Oat straw	14.2[a]	±1.78	25.6[b]	±3.24	39.2[c]	±3.48
Carex	36.8[a]	±4.35	43.5[a]	±2.66	43.2[a]	±2.73
Kentucky bluegrass	35.6[a]	±2.34	43.0[a]	±1.84	44.7[a]	±1.80
Brome-fescue	61.9[a]	±3.48	64.5[a]	±1.73	61.0[a]	±0.93
Pooled data	37.1[a]	±2.63	44.1[b]	±2.11	47.0[b]	±1.59

[ab]Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Discussion

The fact that the celluloses in oat straw, *Carex* and Kentucky bluegrass were digested significantly less than brome-fescue cellulose by each source of inoculum, suggests that the cellulose of brome-fescue was *per se* more digestible than those of oat straw, *Carex* and Kentucky bluegrass. Oat straw cellulose was significantly less digestible than either *Carex* or Kentucky bluegrass cellulose with *Carex* or Kentucky bluegrass inoculum but with brome-fescue inoculum the digestibility of oat straw cellulose was similar to that of the test forage celluloses.

Baumgardt *et al.* (1962) established the regression equation $Y = 31.8 + 0.71X$, where Y = *in vivo* digestibility of dry matter and X = *in vitro* cellulose digestibility. When the *in vitro* digestibility values of *Carex* cellulose with *Carex* inoculum, 36.8 percent, and Kentucky bluegrass cellulose with Kentucky bluegrass inoculum, 43.0 percent, were substituted in the above regression equation, the predicted *in vivo* dry matter digestibilities of *Carex* and Kentucky bluegrass were 58.0 and 61.4 percent, respectively. The predicted dry matter digestibilities of brome-fescue from the digestibility of brome-fescue cellulose *in vitro* with brome-fescue inoculum, 61.0 percent, is 75.2 percent.

Digestion Rate Studies

Introduction

The effective nutritive value of a forage is related to the level of its voluntary intake when it constitutes the entire ration (Crampton, 1957). Balch and Campling (1962) suggested that physical distention of the ruminoreticulum was an important factor regulating the intake of forages. Blaxter and Wilson (1962) and Conrad et al. (1964) observed increased forage intakes as digestibility increased.

The nutritive value index proposed by Crampton et al. in 1960 equates the forage quality to the product of the energy digestibility and relative voluntary consumption. Donefer et al. (1960) reported that percent cellulose digestion after 12 hr incubation in vitro was highly correlated with the nutritive value index ($r = +0.91$). Johnson et al. (1962) confirmed that relative intake could be predicted from data for cellulose digestion after incubation for 12 hours. The theoretical nutritive value indexes of brome-fescue and of the test forages were calculated from the 12 hr in vitro cellulose digestion data.

Experimental

The digestion rates for dry matter and cellulose of Carex, Kentucky bluegrass and brome-fescue were determined by the two-stage in vitro fermentation technique of Tilley and Terry (1963) described previously. Each forage sample was inoculated with strained rumen fluid from an animal fed that particular feed. Random fermentation tubes of each substrate were withdrawn at intervals during the rumen fluid fermentation stage. At each sampling time, three tubes were removed, the bacterial activity checked by the addition of 1 ml of 5 percent $HgCl_2$ and the tubes placed in a cold room at -3°C. When the last

samples had been taken, the frozen samples were thawed and all the tubes centrifuged for 15 min at 1,500 X g. The supernatant fluid was poured off and the pepsin solution added for the second stage of incubation.

The experiment was repeated twice. In the first trial, samples were taken after 8, 16, 24, 32, 40 and 50 hr. In an attempt to determine the length of the lag phase, samples in the second trial were withdrawn after 6, 12, 21, 30, 39 and 48 hr.

Graphs illustrating the rates at which dry matter and cellulose were digested were drawn from the data of each trial. Similar rates were observed in both trials for dry matter and for cellulose digestion. The digestion of dry matter and cellulose at 8, 16, 24, 32, 40 and 48 hr was calculated from these graphs. The average digestion rates for dry matter and cellulose for the two trials are presented in Figures 1 and 2, respectively.

Rate of *In Vitro* Dry Matter Digestion

Results

The average rates at which the dry matter in *Carex*, Kentucky bluegrass and brome-fescue was digested are illustrated in Figure 1. The proportion of brome-fescue digested during the first 8 hr, 48 percent, was higher than that of both *Carex*, 24.8 percent, and Kentucky bluegrass, 26.4 percent. The rates at which the dry matter in carex and Kentucky bluegrass was digested were similar, and did not decline towards the end of the incubation period. The average rate for brome-fescue digestion, 1.62 percent per hr, was higher than that for *Carex*, 1.13 percent per hr, and Kentucky bluegrass, 1.21 percent per hr, over 48 hr.

Discussion

The higher rate of brome-fescue dry matter digestion during the

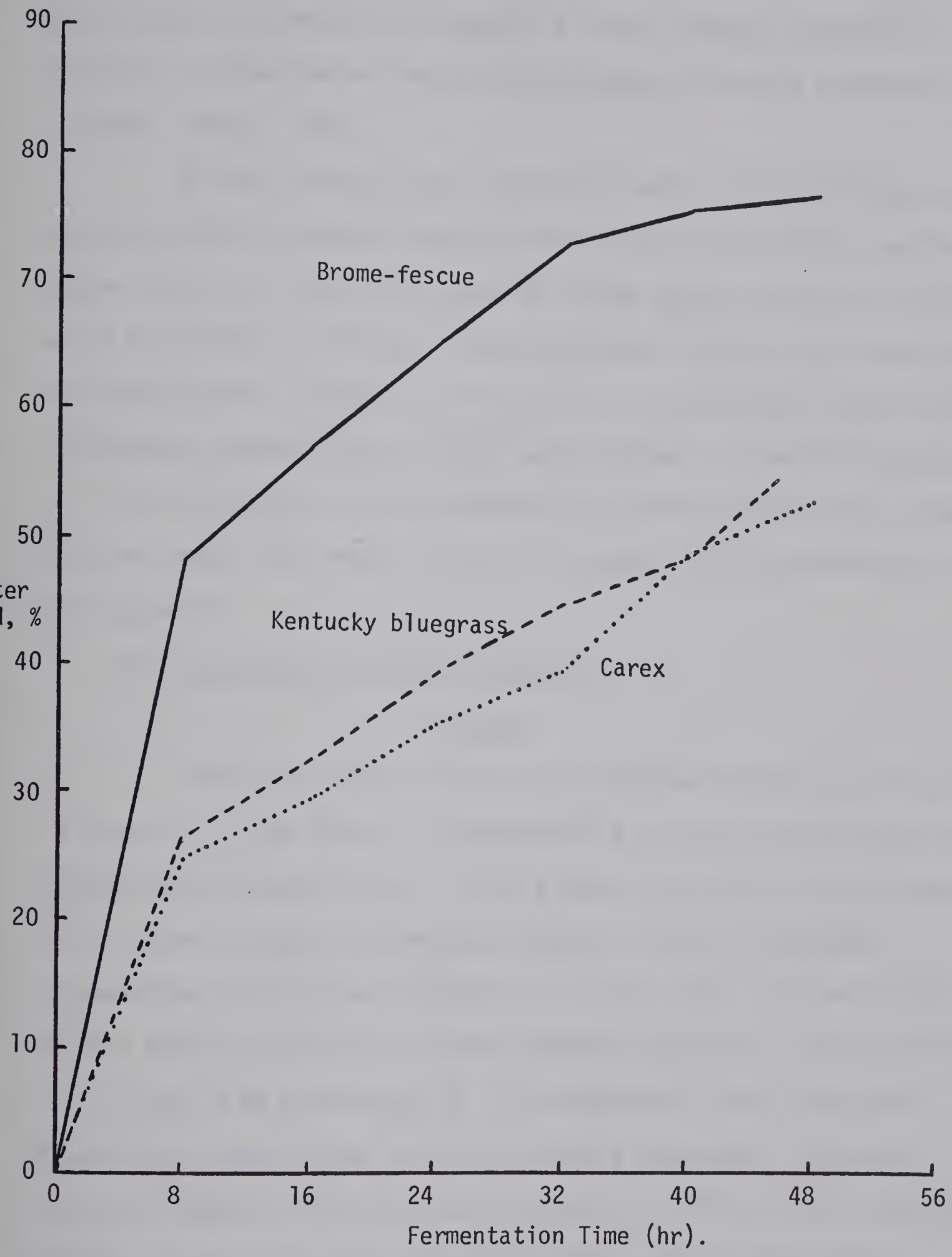

Figure 1. Rate of <u>in vitro</u> dry matter digestion.

early stages of fermentation suggests a larger soluble carbohydrate fraction in brome-fescue than in either Carex or Kentucky bluegrass (Hungate, 1966, p. 228).

On the assumption that higher dry matter digestibilities are associated with increased intakes (Blaxter and Wilson, 1962), considerably higher intakes of brome-fescue than of either Carex or Kentucky bluegrass would be expected. Further, similar voluntary intakes of the test forages would be expected. However, in Experiment 1, considerably higher intakes of Kentucky bluegrass than of Carex were observed. A possible explanation for this difference is that palatability may influence voluntary intake (Marten, 1969); this characteristic of forages is not accounted for in in vitro work.

Rate of In Vitro Cellulose Digestion

Results

The average rates at which cellulose was digested are illustrated in Figure 2. A lag phase of approximately 8 hr was observed during the fermentation of brome-fescue. The lag phase associated with the digestion of cellulose in Carex and Kentucky bluegrass was not pronounced. Brome-fescue cellulose was digested at a faster rate, 1.33 percent per hr than was the cellulose in either Kentucky bluegrass, 0.95 percent per hr or Carex, 0.90 percent per hr. The exponential phase during the digestion of brome-fescue cellulose was more pronounced. Carex and Kentucky bluegrass celluloses were digested at similar rates, though Carex cellulose was a little less digestible after approximately 10 hr.

Discussion

Donefer et al. (1960) reported a significant correlation between the 12 hr in vitro cellulose digestion and the nutritive value

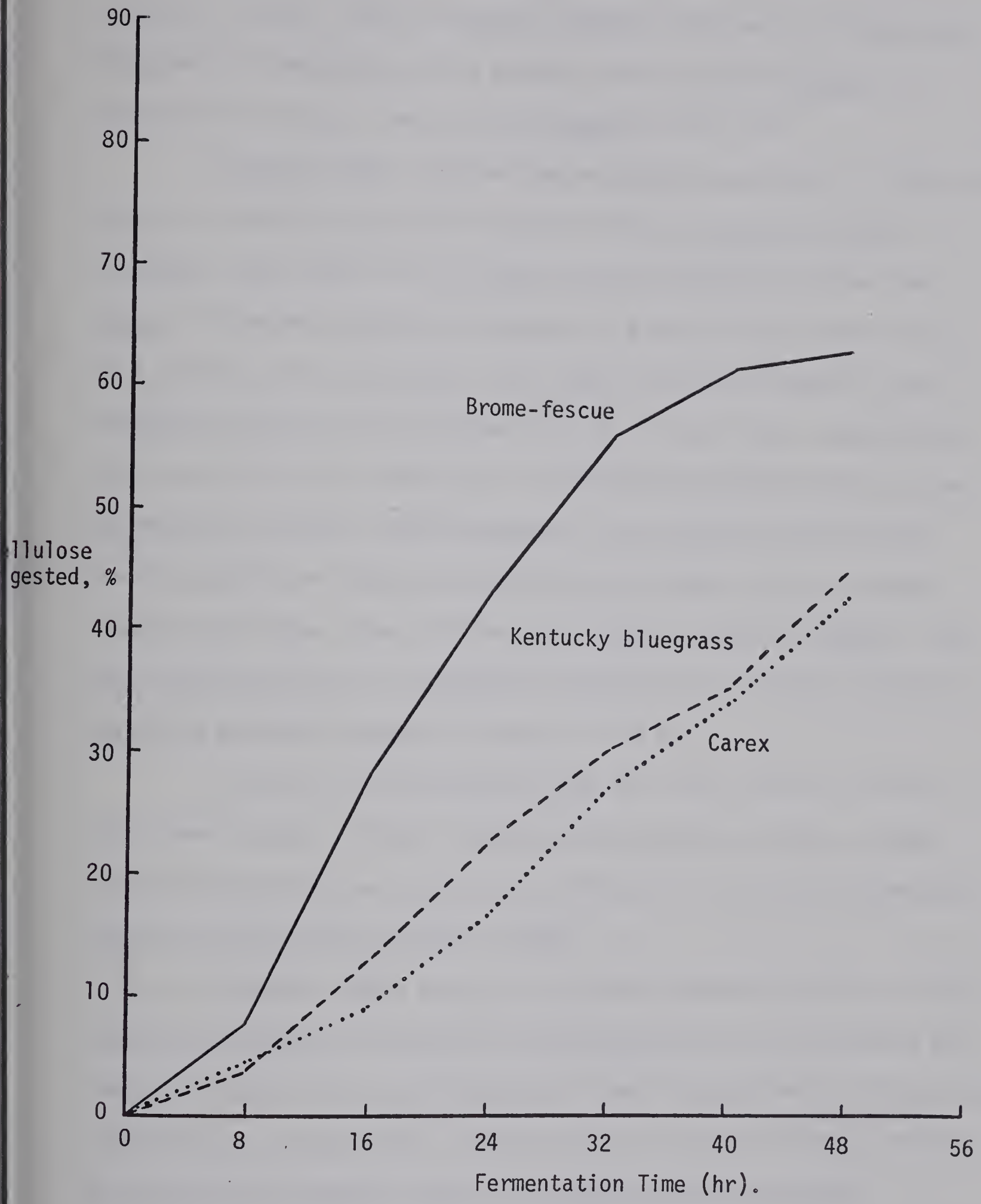

Figure 2. Rate of <u>in vitro</u> cellulose digestion.

index (r = +0.91). Data in Figure 2 indicate that more cellulose was digested in brome-fescue, 17.9 percent, than in either Carex, 6.6 percent, or Kentucky bluegrass, 8.4 percent after 12 hr.

Donefer (1960) reported the regression equation Y = 1.31X - 7.8, where Y = nutritive value index and X = 12 hr in vitro cellulose digestion. When the 12 hr in vitro cellulose digestion values for Carex, 6.6 percent, Kentucky bluegrass, 8.4 percent and brome-fescue, 17.9 percent, are substituted in the above regression equation, the predicted nutritive value indexes are 0.9, 3.2 and 15.3, respectively. These nutritive value indexes are considerably lower than index values determined by Donefer (1960); however, if any significance whatever can be attached to values calculated in this manner, they do suggest a moderately higher index for Kentucky bluegrass than for Carex. Thus the results tend to be in agreement with those for voluntary intake of Carex and Kentucky bluegrass listed in Table 4.

A possible explanation for the low 12-hr digestion values, in the present study, and thus low calculated nutritive values is that strained rumen fluid was used as the inoculant in contrast to phosphate buffer extracts used by Donefer (1960).

Crampton (1960) suggested that the importance of the relative intake of a forage as compared to its digestibility in determining the numerical value of the nutritive value index is approximately 70 percent vs 30 percent, respectively. On this premise, higher relative intakes of the Kentucky bluegrass than of the Carex would be expected.

Experiment 3

Trichloroacetic Acid-Insoluble Nitrogen

Introduction

Bergen et al. (1968) stated that dense populations of rumen microbes are desirable since both the microbial protein and the end products of fermentation play significant roles in ruminant nutrition. Hungate (1966) reported that readily fermentable feeds support denser microbial populations in the rumen than do less digestible substrates. Cline et al. (1958) demonstrated that reliable estimates of bacterial protein in rumen fluid could be made from the amounts of trichloroacetic acid-insoluble nitrogen in the rumen fluid. The purpose of Experiment 3 was to compare the approximate sizes of the microbial populations in the rumen fluid from the cows fed Carex, Kentucky blue-grass and brome-fescue as estimated from amounts of trichloroacetic acid-insoluble nitrogen in the fluid.

Experimental

Samples of rumen fluid were taken from the fluid collected for use in the in vitro digestion studies, Experiment 2, and stored at -3°C for analysis at a later date. Trichloroacetic acid-insoluble nitrogen was determined by the method of Cline et al. (1958) with minor modifications. One ml of warmed 80 percent w/v trichloroacetic acid was added to 7 ml of strained rumen fluid. The suspension was mixed and chilled in ice; after 10 min (in place of 18-24 hr) the suspension was centrifuged at 1,500 X g for 15 min. The supernatant fluid was poured off and the precipitate resuspended in 10 ml of a 10 percent trichloroacetic acid solution. The suspension was re-centrifuged and the precipitate transferred to Kjeldahl flasks for nitrogen analysis (AOAC, 1965). Each sample was analyzed in

triplicate. Comparisons of means were carried out according to the method described by Steel and Torrie (1960).

Results

Significantly higher ($P < 0.05$) amounts of trichloroacetic acid-insoluble nitrogen were present in the rumen fluid from the animal fed brome-fescue than were present in the rumen fluid from the animals fed either Carex or Kentucky bluegrass (Table 9).

Table 9

Trichloroacetic acid-insoluble nitrogen in rumen fluid.

	Diet of donor animal		
	Carex	Kentucky bluegrass	Brome-fescue
TCA-N (mg/ml)	1.57[a]	1.73[a]	5.18[b]

[ab]Means bearing different superscripts differ significantly ($P < 0.05$).

Discussion

On the premise that trichloroacetic acid-insoluble nitrogen represents a reliable estimate of bacterial protein (Cline et al., 1958), the data in Table 9 suggest that the microbial populations present in the rumen fluid from the cow fed brome-fescue were three times as large as those in the fluid from the heifers fed either Carex or Kentucky bluegrass.

Readily fermentable substrates support higher microbial populations in the rumen (Warner, 1962) and increased digestion rates are associated with higher voluntary intakes (Crampton et al., 1960).

The average rate of dry matter digestion (p. 33) in brome-fescue, 1.62 percent per hr, was higher than that in either Carex, 1.13 percent per hr, or Kentucky bluegrass, 1.21 percent per hr, and larger microbial populations were expected in brome-fescue rumen fluid than in the test forage rumen fluids. Further, denser populations were expected in the rumen fluid from the animals fed Kentucky bluegrass, with intakes of 8.9 kg/day, than in the rumen fluid from the animals fed Carex, with intakes of 5.6 kg/day.

This hypothesis is not supported by the results in the present study, in which the voluntary intakes were not related to trichloroacetic acid-insoluble nitrogen.

Greater amounts of microbial protein and fermentation products would be produced on diets of brome-fescue than on diets of Carex or Kentucky bluegrass. The production of VFA is discussed in Experiment 5.

Experiment 4

Water Extracts of Forages

Introduction

Marquardt and Asplund (1964) suggested that the water soluble fraction represents that portion of the forage most available to the microbial population. They hypothesized that the ability of this fraction to support cellulose digestion by rumen microorganisms would indicate the nutritive value of the forage. However, since wide difference in *in vitro* cellulose digestion were required to measure differences in nutritive value, this method was not considered sensitive enough for routine forage evaluation. They also investigated the response in cellulose digestion by rumen microbes to supplementation of forage extracts with nitrogen, phosphorus and sulphur. They reported that the addition of phosphate and sulphate in combination resulted in a marked increase in cellulose digestion, but that these ions singly had little effect. The response to urea supplementation was variable. A modification of this method was used in the present work to investigate the ability of test forage and brome-fescue extracts to support the digestion of purified cellulose by rumen bacteria.

Experimental

Forage Extracts

Water extracts of forages were prepared by the procedure described by Marquardt and Asplund in 1964. Samples of *Carex*, Kentucky bluegrass and brome-fescue were dried in a forced-draft oven at 100°C for 24 hr and ground in a Christy and Norris 8-in laboratory mill fitted with a 1 mm screen. The forages were suspended in 20 times their weight of water and allowed to stand for 3 hr at room temperature before filtration

through a No. 4 Whatman filter paper. The filtrates will hereinafter be referred to as extracts. The extracts were stored in glass containers at 4°C until required. As a sediment precipitated during storage the extracts were re-filtered prior to use.

Inoculum Preparation

Partially washed cell preparations were used in this experiment in contrast to the phosphate buffer extracts used by Marquardt and Asplund (1964). Rumen ingesta were collected from a fistulated Hereford heifer fed a diet of Kentucky bluegrass. The heifer had free access to calcium phosphate (20 percent phosphorus and 18.5 percent calcium) and iodized salt in the ratio of 1:1. Water was available ad libitum from an automatic water bowl.

Ingesta from different parts of the rumen was squeezed through four layers of cheese-cloth in a squeeze press. The expressed liquor was filtered through 12 layers of cheese-cloth and collected in a large thermos flask. On arrival at the laboratory, the fluid was centrifuged at 16,000X g for 20 min at 15°C. The supernatant fluid was discarded and the residue re-suspended in a buffer solution in a Waring blender. The buffer-inoculum suspension was filtered through 12 layers of cheese-cloth to remove any large particles. The buffer solution was prepared by dissolving 10 g of Na_2CO_3 in one litre of de-ionized water. Anaerobic conditions were maintained by bubbling a stream of CO_2 through the suspension and the temperature was held at 39°C where possible.

In Vitro Fermentation

The digestion of purifed cellulose[1] in vitro was determined by the first stage of the in vitro technique developed by Tilley and Terry

[1]Solka Floc SW 40 A, obtained from Brown Co., Berlin, N.H.

(1963), with some modifications. Samples of purified cellulose (200 mg) were accurately, 0.1 mg, weighed into 90 ml polypropylene centrifuge tubes. The final volume in each tube was 50 ml and contained the following:

1) buffer-inoculum suspension, containing the residue of 50 ml of strained rumen fluid plus 200 mg of Na_2CO_3, - 20 ml
2) a solution containing 63 mg of urea and mineral supplements (when added), and - 10 ml
3) aqueous extract equivalent to 1 g of forage (in the control tubes, de-ionized water was added instead). - 20 ml

The fermentation mixture in each tube was flushed out with CO_2 and the tubes were then sealed with a rubber stopper fitted with a Bunsen gas release valve. After sealing, the tubes were incubated at 38°C in the dark for 24 hr.

Determination of Cellulose Digestion

At the end of the incubation period the tubes were centrifuged at 1,500 X g for 20 min. The supernatant fluid was discarded and the residue dried in a vacuum oven at 95°C for 48 hr. The dry weight of the residue was determined. Total dry matter in the blank tubes, which represented microorganisms and undigested food particles from the rumen liquor, was determined after incubation. In vitro cellulose digestion was calculated as the percent dry matter disappearance during fermentation.

$$\text{Cell. dig., \%} = 100 - \frac{(\text{cell. before dig.} + \text{DM in blank} - \text{DM after dig.}) \times 100}{\text{cell. before dig.}}$$

Analysis of variance and determination of standard errors were carried out according to the methods described by Steel and Torrie (1960). The computations were done on an Olivetti-Underwood Programma 101.

Experiment 4a

The purpose of the first experiment was to investigate the abilities of the three forage extracts to support the *in vitro* digestion of purified cellulose by rumen microbes. Aqueous extracts of 1 g of forage plus 63 mg of urea, buffered with 200 mg of Na_2CO_3, were used as the media for the fermentation of 200 mg of purified cellulose by rumen microorganisms. Twenty ml of de-ionized water were added to the control tubes in place of extracts. Each fermentation was in quadruplicate and the trial repeated twice.

Results

The results for this experiment are given in Table 10. The digestion of purified cellulose was increased significantly ($P < 0.05$) by the addition to the media of Kentucky bluegrass and brome-fescue extracts in Trial 1, and by the addition of *Carex*, Kentucky bluegrass and brome-fescue extracts in Trial 2. In both trials 1 and 2, the greatest response was observed with the addition of Kentucky bluegrass extract, 1.92 and 3.92 times, respectively, and the smallest response when *Carex* extracts were added, 1.19 and 2.32 times, respectively.

Table 10

Percent cellulose digested as affected by forage extracts.

Trial	Forage extracts							
	Control		*Carex*		Kentucky bluegrass		Brome-fescue	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	29.1^{a}	±2.34	34.7^{a}	±1.31	56.0^{b}	±3.47	39.7^{c}	±0.60
2	15.2^{a}	±1.97	35.3^{b}	±8.60	59.5^{c}	±7.81	56.6^{c}	±1.84

^{abc}Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Discussion

The results of Experiment 4a indicate that factors were present in the forage extracts that enhanced the digestion of purified cellulose by partially washed rumen microorganisms. The addition of Kentucky bluegrass extracts to the fermentation media increased cellulose digestion more than did the addition of *Carex* extracts. This suggests that the Kentucky bluegrass extract was a richer source of those factors that enhanced cellulose digestion than was *Carex* extract.

The digestion of cellulose by rumen microbes was enhanced to an equal or greater extent by Kentucky bluegrass extracts than by brome-fescue extracts. This was surprising in that, as has been indicated, the brome-fescue appeared to be clearly superior to the Kentucky bluegrass in nutritive value and it was therefore expected to have a superior water soluble fraction. These responses will be discussed later in the General Discussion.

Experiment 4b

Results of Experiment 4a indicated that the digestion of purified cellulose by partially washed rumen bacteria was enhanced by the addition of aqueous extracts of forages; the response was not the same with all extracts. Marquardt and Asplund (1964) reported that adequate amounts of phosphorus, sulphur and nitrogen for the cellulolytic rumen microorganisms were not always present in aqueous extracts of forages. Chemical analysis of the forages (Table 3) indicated lower levels of phosphorus in both Carex, 0.05 percent, and Kentucky bluegrass, 0.06 percent, than in brome-fescue, 0.13 percent. In Experiment 4b, the effects of adding phosphate-sulphate supplement to forage extract media were studied.

Experimental

The aqueous extracts of 1 g of forage plus 63 mg of urea, buffered with 200 mg of Na_2CO_3, were used as the basal media for the fermentation of 200 mg of purified cellulose by rumen microbes. One series of tubes was charged with forage extracts alone. To the extracts in each tube of another series was added a mixture of 58 mg of Na_2HPO_4, 42 mg of NaH_2PO_4 and 10 mg of Na_2SO_4. This is hereinafter referred to as phosphate-sulphate. Extracts were not added to either set of control tubes; phosphate-sulphate was added to one set of control tubes, but not to the second set. Each fermentation was in triplicate and the trial was repeated twice.

Results

Cellulose digestion in the controls without phosphate-sulphate was significantly different from that in the supplemented controls (Table 11). Supplementation of Carex and Kentucky bluegrass extracts with

phosphate-sulphate improved cellulose digestion significantly ($P < 0.05$) in all but the second trial with Kentucky bluegrass extract. The greatest response in cellulose digestion to treatment with phosphate-sulphate was observed with Carex extract, 3.64 and 1.32 times, in trials 1 and 2, respectively.

Table 11

Percent cellulose digested as affected by forage extracts and phosphate-sulphate supplementation

Forage extract	Trial	Treatments	
		None	Phosphate-sulphate
		Mean SE	Mean SE
Control	1	$8.3^{a}\pm0.43$	$4.9^{b}\pm0.18$
	2	$10.1^{a}\pm1.66$	$22.7^{b}\pm2.91$
Carex	1	$5.9^{a}\pm1.00$	$21.5^{b}\pm0.59$
	2	$46.8^{a}\pm5.18$	$61.6^{b}\pm2.21$
Kentucky bluegrass	1	$22.2^{a}\pm0.63$	$42.1^{b}\pm0.35$
	2	$68.0^{a}\pm3.77$	$71.5^{a}\pm1.55$
Brome-fescue	1	$24.6^{a}\pm0.44$	$28.6^{a}\pm2.12$
	2	$61.2^{a}\pm5.16$	$61.1^{a}\pm3.44$

^{ab}Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Discussion

No satisfactory explanation can be given for the fact that the digestion of cellulose in the control tubes was inhibited in trial 1 and increased in trial 2 by the addition of phosphate-sulphate.

The responses with Kentucky bluegrass were again equal to or greater than those with brome-fescue. This aspect will be discussed in relation to the experimental data in the General Discussion.

Experiment 4c

Results in Experiment 4b indicated that when phosphate-sulphate was added to media composed basically of extracts of Carex or Kentucky bluegrass, cellulose digestion by rumen microorganisms was increased. The purpose of Experiment 4c was to compare the effects on cellulose digestion of phosphorus and sulphur when added singly and in combination to forage extract media.

Experimental

The aqueous extracts of 1 g of forage plus 63 mg of urea, buffered with 200 mg of Na_2CO_3, were used as the basal media for the fermentation of 200 mg of purified cellulose by rumen microorganisms. The basal medium was added to four series of tubes. The tubes in each series were supplemented with one of the following treatments.

i) None

ii) 58 mg of Na_2HPO_4 + 42 mg of NaH_2PO_4 (phosphate)

iii) 10 mg of Na_2SO_4 (sulphate)

iv) phosphate-sulphate

Four series of control tubes were treated in the same manner with the exception that forage extracts were not added to the fermentation tubes. Each fermentation was in triplicate and the trial repeated twice.

Results

Cellulose digestion was not significantly ($P < 0.05$) affected by any of the treatments in either the control tubes or brome-fescue extracts (Table 12). When Carex and Kentucky bluegrass extracts were treated with phosphate, cellulose digestion was increased significantly. Phosphate-sulphate increased the digestion of cellulose with Carex and Kentucky bluegrass extracts at the 5 percent level of significance in all

but trial 2 with Kentucky bluegrass extract. With the exception of Carex extract in trial 1, sulphate supplementation had no significant effect on cellulose digestion.

Table 12

Percent cellulose digested as affected by forage extracts and treatments with phosphate and sulphate.

Forage extract	Trial	Treatments							
		None		Sulphate		Phosphate		Phosphate-sulphate	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	1	17.3^{a}	±1.42	17.7^{a}	±0.99	18.1^{a}	±1.74	15.6^{a}	±1.56
	2	14.2^{a}	±1.44	13.7^{a}	±1.94	12.4^{a}	±2.17	12.6^{a}	±1.33
Carex	1	16.2^{a}	±2.00	31.6^{b}	±2.63	57.6^{c}	±0.35	50.8^{c}	±4.12
	2	10.0^{a}	±0.54	10.6^{a}	±1.14	36.6^{b}	±2.90	25.2^{c}	±3.75
Kentucky bluegrass	1	48.9^{a}	±2.62	50.6^{a}	±3.63	60.8^{b}	±6.25	57.4^{b}	±1.14
	2	35.0^{a}	±1.05	29.1^{a}	±2.80	43.3^{b}	±0.05	39.6^{a}	±1.10
Brome-fescue	1	47.5^{a}	±8.28	50.7^{a}	±2.64	46.7^{a}	±1.52	49.4^{a}	±4.10
	2	37.8^{a}	±2.35	38.6^{a}	±0.10	37.7^{a}	±2.35	36.3^{a}	±1.80

^{abc}Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Discussion

The addition of phosphate to Carex and Kentucky bluegrass extracts tended to result in larger increases in cellulose digestion than did phosphate-sulphate supplementation; the addition of sulphate alone was without effect in all but trial 1 with Carex. This suggests that

under the conditions of this experiment, insufficient amounts of phosphate were present in the aqueous extracts of both 1 g of Carex and 1 g of Kentucky bluegrass to support optimum digestion of 200 mg of purified cellulose by cellulolytic rumen bacteria. The digestion of cellulose by rumen microbes with brome-fescue extract was not influenced by any of the treatments. Again, the fact that the digestion of cellulose was enhanced equally or more by the addition of Kentucky bluegrass extract than it was by the brome-fescue extract will be discussed in the General Discussion.

In the present study, the cellulolytic activities of the inocula differed between trials. Marquardt and Asplund (1969) reported a similar finding.

Experiment 5

Volatile Fatty Acid Production

Introduction

Von Tappeiner (1882), as cited by Marston (1948), reported the production of gases and fatty acids during the fermentation of cellulose by rumen microorganisms. In 1942, Phillipson emphasized the importance of VFA in the nutrition of ruminants and suggested that the composition of the diet affected the rumen population and pattern of VFA in the rumen.

The fact that rumen fluid volume varies (Alexander et al., 1969) is one factor which may affect the reliability of VFA concentration in rumen fluid as an indicator of total VFA production in the rumen. However, Weller et al. (1969), using an isotope technique, reported that despite irregular feed intake, with water ad libitum, good predictions of mean VFA production rates could be made from mean VFA concentrations in rumen fluid. Asplund et al. (1958) compared the total VFA production from 11 hays in all-glass fermentation vessels; total VFA concentrations in the rumen fluid from sheep fed hay and from sheep fed straw were also compared. The results suggested a close relationship between total VFA and dry matter digestibility in vivo. Higher fatty acid levels were present in the rumen fluid from sheep fed hay than from sheep fed straw.

Experimental

Samples of rumen fluid were collected from each of the cows fed Carex, Kentucky bluegrass or brome-fescue; the samples were obtained at the same time as those collected for the in vitro digestibility studies reported earlier. Six samples of rumen fluid from each cow were therefore collected at intervals of at least one week. The samples were

stored in polypropylene bottles at -3°C until analysed.

The rumen fluid was prepared for VFA analysis by the method outlined by Balwani et al. (1969). The samples were thawed at room temperature and 2 ml of 25 percent meta-phosphoric acid were immediately added to 8 ml of rumen fluid and the mixture allowed to stand for 15 min. The samples were then centrifuged at 2,000X g for 15 min and the supernatant fluid analysed by gas-liquid chromatography (GLC).

Total and individual concentrations of VFA in rumen fluid samples were determined by GLC using a model 600-D Aerograph HY-F1 GLC with a flame ionization detector. A 20 µl sample of the prepared supernatant fluid was injected directly into an aluminum column (6 ft X 1/4 inch) packed with a commercial preparation of 150-200 Porapak Q. Helium was used as the carrier gas at a flow rate of 102 ml/min and hydrogen was supplied to the detector at a flow rate of 32 ml/min. An injection temperature of 262°C and an oven temperature of 224°C were used throughout. A flame setting of 1 was maintained and attenuations of 8, 16 and 32 were used as required.

Analysis of variance, mean comparisons and standard error determinations were carried out according to the methods described by Steel and Torrie (1960). The computations were done on an IBM 360/67 computer.

Results

Significantly higher concentrations of total VFA were present in the rumen fluid from the cow fed brome-fescue, 11.17 m-equiv /100 ml, than in the rumen fluid from heifers fed either Carex, 8.65 m-equiv / 100 ml, or Kentucky bluegrass, 8.76 m-equiv /100 ml.

Mean percentages of individual VFA present in the rumen fluid from the animals fed brome-fescue and test hays are presented in Table 13. In the rumen fluid from the cow fed brome-fescue, lower concentrations of acetic acid were found, whereas the concentrations of iso-butyric, n-butyric and iso-valeric acids tended to be higher than those present in the rumen fluid from the heifers fed Carex or Kentucky bluegrass.

Table 13

Total and mean percent VFA in rumen fluid from cows fed Carex, Kentucky bluegrass and brome-fescue.

VFA	Diet					
	Carex		Kentucky bluegrass		Brome-fescue	
	Mean	SE	Mean	SE	Mean	SE
Total (m-equiv /100 ml)	8.65[a]		8.76[a]		11.17[b]	
Acetic, %	72.7[a]	±1.02	72.4[a]	±0.75	67.5[b]	±1.63
Propionic, %	15.4[a]	±1.16	13.6[a]	±0.82	14.3[a]	±0.83
Iso-butyric, %	0.75[a]	±0.00	1.04[a]	±0.08	1.61[b]	±0.13
n-butyric, %	9.01[a]	±0.41	9.79[a]	±0.27	11.69[a]	±1.20
Iso-valeric, %	1.14[a]	±0.05	1.71[ab]	±0.16	2.31[b]	±0.21
n-valeric, %	1.12[a]	±0.04	1.66[a]	±0.22	1.48[a]	±0.12

[ab]Means within each row bearing different superscripts differ significantly ($P < 0.05$).

Discussion

Relatively high proportions, 67-73 percent, of acetic acid were present in the rumen fluid from animals fed diets of Carex, Kentucky bluegrass, or brome-fescue. This agrees with results published by Bath and Rook (1965), who found that all roughage diets showed high proportions of acetic acid, about 70 percent. Chalupa and McCullough (1967) reported that higher proportions of acetic acid were associated with lower digestibility. From this premise it may be theorized that brome-fescue was more digestible than either Carex or Kentucky bluegrass. The digestibilities of Carex and Kentucky bluegrass were very similar.

Certain short-chain fatty acids are required as growth factors for cellulolytic rumen microorganisms (Bentley et al., 1954, 1955). These and other workers suggested that one or more of the volatile fatty acids, such as n-valeric, iso-valeric, 2-methylbutyric and iso-butyric, were essential for the growth of some species. Significantly higher levels of iso-butyric acid were found in brome-fescue rumen fluid than in the fluid from either of the animals fed the test forages. Further, the highest levels of iso- and n-butyric and iso-valeric acids were present in brome-fescue rumen fluid.

Though the amounts of iso- and n-butyric and iso- and n-valeric acids in the test forage / rumen fluids did not differ significantly, higher concentrations of these acids were found in the rumen fluid from the heifer fed Kentucky bluegrass. The trend was consistent, and the differences may have had biological significance, with the lower levels related to less prolific and less active cellulolytic populations. The values for trichloroacetic acid-insoluble nitrogen (Experiment 3) found in rumen fluid from animals fed Carex, 1.57 mg/ml, Kentucky bluegrass, 1.73 mg/ml,

and brome-fescue, 5.18 mg/ml, support this possibility. Further, the ability of brome-fescue inocula to digest cellulose, 47.0 percent, was superior to that of Kentucky bluegrass inocula, 44.1 percent; which in turn was more active than that of Carex inocula, 37.1 percent, (pooled data, Table 8).

Asplund et al. (1958) observed significant correlations between total VFA production and dry matter digestibility. On this basis brome-fescue, 11.7 m-equiv VFA/100 ml, would be predicted to be more digestible than either Carex, 8.65 m-equiv VFA/100 ml, or Kentucky bluegrass, 8.76 m-equiv VFA/100 ml.

Weller et al. (1969) stated that ruminal VFA concentrations represented a pasture's full potential yield of VFA to the animal and so indicated its nutritive value. In terms of this reference, the theoretical nutritive value of brome-fescue was higher than that of either Carex or Kentucky bluegrass; the latter two being similar.

General Discussion

The test forages were harvested before maturity when forage quality is relatively high. At maturity, protein levels are generally lower and the proportion of cellulose increases (Sullivan, 1969).

Samples of hays harvested at later stages of growth from similar areas to the test forages in the lake bottom in 1968 were analysed in the laboratory and the results are presented in Appendix 2, Table 14. Additional feed analysis data for forage samples from similar areas in the lake bottom area were obtained from the Agricultural Soil and Feed Testing Laboratory (Appendix 3, Table 15).

Protein contents of mixed hays (Kentucky bluegrass?) harvested in 1968, 8.7, 9.6 and 9.9 percent, and in samples from the Agricultural Soil and Feed Testing Laboratory, swamp grass (Carex?), 7.8 percent, and mixed hay (Kentucky bluegrass?), 7.5 percent, were all lower than those in the test forages, Carex, 11.2 percent, and Kentucky bluegrass, 11.5 percent (Table 2, p. 15). This additional information suggests that Carex and Kentucky bluegrass harvested from the lake bottom at later stages of growth would not have met the protein requirements of all classes of beef cattle, which range from 5.9 percent for mature cattle to 10.0 percent for 441 lb growing steers gaining 0.55 lb per day (NRC, 1970).

The Agricultural Soil and Feed Testing Laboratory determined crude fibre but not cellulose. Crude fiber is present in quantities close to those of cellulose, though these two are not of the same composition - crude fibre containing some lignin and having lost some cellulose (Sullivan, 1964). Comparison of the data in Appendix 3, Table 15 and those in Table 2, p. 15, shows that the crude fiber content of the swamp grass (Carex?) samples analysed in the Agricultural Soil and Feed

Testing Laboratory was 1.2 times as high as was the cellulose content of the Carex hay used in the present study; similarly the average crude fiber level for 'mixed grass' and '85 percent fine hay' (Kentucky bluegrass?) was 1.3 times that for cellulose in the test Kentucky bluegrass. Further, the data in Appendix 2, Table 14, show that the mean cellulose content of the 3 samples of mixed hay (Kentucky bluegrass?) harvested in 1968 was 1.2 times that for cellulose in the test Kentucky bluegrass (Table 2). Insofar as percent cellulose and dry matter digestibility are negatively correlated ($r = -0.72$, Sullivan, 1964), higher cellulose contents at later stages of growth would be associated with lower digestibilities.

The levels of phosphorus in mixed hays (Kentucky bluegrass?) harvested in 1968 ranged from 0.06 to 0.08 percent; similar levels were found in the test forages, Carex, 0.05 percent, and Kentucky bluegrass, 0.06 percent. Values obtained from the Agricultural Soil and Feed Testing Laboratory were a little higher in all samples and ranged from 0.10 to 0.13 percent. The phosphorus requirements for beef cattle (NRC, 1970), range from 0.18 percent for 441 lb growing steers gaining 0.55 lb per day to 0.16 percent for mature cattle. Both the analytical data for the feeds and the results of the experiments dealing with forage extracts (Experiment 4c) indicate that the phosphorus requirements of beef cattle and of cellulolytic bacteria would not have been met by the test forages alone.

Large differences were observed in the calcium levels in the test forages, Carex, 0.44 percent, and Kentucky bluegrass, 0.18 percent (Table 2). Data from the Agricultural Soil and Feed Testing Laboratory, Appendix 3, Table 15, indicate that the levels of calcium in swamp grass (Carex?), 0.32 and 0.72 percent, and in mixed grass (Kentucky bluegrass?),

0.25 and 0.36 percent, were similar to the amounts found in the mixed hays (Kentucky bluegrass?), 0.32 to 0.36 percent, harvested in 1968 (Appendix 2, Table 14). These data suggest that the level of calcium in the Kentucky bluegrass investigated in the present study was not typical of forages from similar areas in the lake bottom.

The protein content of forages is positively correlated to dry matter digestibility (Asplund et al., 1958) and the cellulose content is negatively correlated to dry matter digestibility (Sullivan, 1964). Further, Blaxter and Wilson (1962) observed that dry matter digestibility was related to voluntary intake. As similar levels of protein and of cellulose were found in Carex, and in Kentucky bluegrass, the levels of these constituents in the test forages did not explain the fact that voluntary consumption (Table 4, p. 20) of Kentucky bluegrass was 1.6 times that of Carex.

The observed and predicted digestibilities of dry matter in the test forages were similar in all cases, though there was a consistent trend for Kentucky bluegrass dry matter to be more digestible than that of Carex. Similar intakes of the test forages were estimated though, in every case, slightly higher intakes of Kentucky bluegrass were predicted. In vitro and predicted digestibilities and intakes of brome-fescue were always higher, and of oat straw always lower, than those of the test forages.

The in vivo dry matter digestibilities predicted from in vitro dry matter and cellulose digestion experiments (p. 28, 31) were in agreement with the data obtained from the digestibility trial (Table 4, p. 20). Greenhalgh and Reid (1967) reported a positive association between palatability and intake but found no relationship between palatability

and digestibility. Thus, it may be suggested that differences in palatability were responsible for the large differences in voluntary consumption, but that these differences in forage acceptability were not related to the digestibility of the forages.

Readily digestible substrates tend to support higher rumen microbial populations than do less digestible substrates (Warner, 1962). Trichloroacetic acid-insoluble nitrogen provides a reliable estimate of bacterial protein and of bacterial numbers (Cline, et al., 1958). Thus the fact that similar levels of trichloroacetic acid-insoluble nitrogen were found in the rumen fluids of animals fed Carex or Kentucky bluegrass (Table 9, p. 39) is in agreement with the observed similarity in digestibility values for the test forages in vivo (Table 4, p. 20) and in vitro (Tables 5 and 7, p. 27 and 30).

In all the experiments dealing with forage extracts (Experiments 4a, b, c), the digestion of purified cellulose by rumen microorganisms was enhanced to an equal or greater extent by the addition of Kentucky bluegrass extract than by the addition of brome-fescue extract. Marquardt and Asplund (1964) suggested that the forage extracts contained the soluble fraction most readily available to the rumen population and that the ability of this fraction to enhance the digestion of purified cellulose by rumen microbes provided an estimate of its size and nutritive value. Assuming that the rate of digestion during the first few hours of incubation indicates the amount of soluble carbohydrates in the substrate (Hungate, 1966), Figure 1 (p. 34) suggests that a considerably larger soluble fraction was present in brome-fescue than in either test forage. From the data in Table 2 it is evident that brome-fescue contained appreciably more protein and ash and appreciably less cellulose than did

either Carex or Kentucky bluegrass, suggesting that brome-fescue might provide a larger water soluble fraction. Thus, a considerably larger response in the digestion of cellulose by rumen microbes was expected with the addition of brome-fescue as compared to Kentucky bluegrass extract; a satisfactory explanation for th fact that differences, if any, were in favor of Kentucky bluegrass is not apparent.

Significant correlations have been observed between total VFA production and dry matter digestibility (Asplund et al., 1958). The highest total VFA concentrations were found in brome-fescue rumen fluid, 11.17 m-equiv/100 ml. Similar VFA levels were present in the rumen fluid from the heifers fed Carex, 8.65 m-equiv/100 ml, and in that from heifers fed Kentucky bluegrass, 8.76 m-equiv/100 ml (Experiment 3). Data from the analysis of VFA in rumen fluid (Table 13) illustrate a consistent trend for higher proportions of those fatty acids required for growth by cellulolytic rumen bacteria to occur in Kentucky bluegrass rumen fluid than in Carex fluid. The highest levels of all but one of these fatty acids were found in the rumen fluid from the cow fed brome-fescue. It is quite probable that these differences may have had biological significance and that conditions in the rumens of the animals fed Kentucky bluegrass were move favourable for the growth of cellulolytic microorganisms than were the conditions in the rumens of those heifers fed Carex.

Summary and Conclusions

The phosphorus levels in the test forages did not meet the nutrient requirements of beef cattle. Further, the phosphorus requirements of cellulolytic rumen microorganisms were not supplied by aqueous extracts of the test forages. No evidence of trace mineral deficiency or excess was obtained.

The voluntary intake of Kentucky bluegrass was 1.6 times that of Carex.

The cellulose and dry matter of Kentucky bluegrass were more digestible than were those of Carex. The digestibilities of these constituents of brome-fescue were higher, and of oat straw lower, than those of the test forages.

The pattern of volatile fatty acids in the rumen fluid of the cows fed Kentucky bluegrass may have been more favourable for the growth of cellulolytic rumen microbes than that of those found in the rumen fluid of the cows fed Carex. The pattern of fatty acids in the rumen fluid of the cow fed brome-fescue would be expected to favour the highest populations of cellulolytic microorganisms.

On the basis of results obtained in the present study, it is suggested that the quality of Kentucky bluegrass was higher than that of Carex.

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Appendix 1

Rumen Fistulation

The Hereford heifers were fistulated by Dr. A.J.F. Webster, Department of Animal Science, by a modification of the technique described by Balch and Cowie in 1962. The operation was carried out under para-vertebral analgesia of the last thoracic and first three lumbar sequential nerves using Novocain. Analgesia was reinforced by local application of Novocain when necessary.

A circular incision, having the dimensions of the stem of the cannula, was made through the left flank, and the abdominal muscles were separated by blunt dissection. The surface of the rumen was drawn up into the incision and sutured with 0-gauge chromic gut in two layers to (1) the peritoneum and transversus abdominis muscle, and (2) the skin of the flank. The wound was dressed and covered.

After 10 days the exposed rumen wall, which had become dry and avascular, was removed and the cannula inserted. The fistula was cleaned regularly and dressed with a sulphonamide powder.

The cannulae and inflatable bungs were manufactured by the Avon India Rubber Company, Melksham, Wilts., England. The bungs were easy to remove and caused a minimum discomfortto the animals.

Appendix 2

Table 14

Composition of hays harvested from the Cormie Ranch Ltd. lake bottom in 1968.

	Mixed hay 1 (Kentucky bluegrass?)	Mixed hay 2 (Kentucky bluegrass?)	Mixed hay 3 (Kentucky bluegrass?)
Moisture, %	8.3	8.2	8.6
Protein, %	9.6	9.9	8.7
Cellulose, %	34.4	33.5	34.8
Ash, %	6.4	6.2	7.1
P, %	0.08	0.08	0.06
Ca, %	0.36	0.32	0.35
Mg, %	0.16	0.19	0.12
K, %	1.41	1.02	1.58
Cu, ppm	7.4	6.9	7.6
Mn, ppm	188	250	99
Zn, ppm	64.3	32.5	38.4
Fe, ppm	86.7	87.4	145.5

Appendix 3

Table 15

Data supplied by the Agricultural Soil and Feed Testing Laboratory, Alberta Department of Agriculture on hays grown on the Cormie Ranch Ltd.

	Location	Growth stage	Mois-ture	Protein	Crude fibre	Ca	P
			%	% air dry basis	% air dry basis	% air dry basis	% air dry basis
Swamp grass (Carex?) - 100%	NW 18-51-5-W5	mature	8.5	9.6	39.6	0.32	0.12
Swamp grass (Carex?) - 100%	SW 18-51-5-W5	mature	7.5	7.8	36.2	0.72	0.10
Mixed grass (Kentucky bluegrass?)	NW 6-51-5-W5	flowering	9.1	11.2	36.8	0.25	0.13
85% fine hay (Kentucky bluegrass?) 15% coarse hay (Carex?)	SW 18-51-5-W5		15.3	7.5	42.4	0.36	0.11